

AMENDMENTS TO THE SPECIFICATION:

**Please amend the paragraph on page 5, lines 19-28 as follows:**

Figure 1. (A) The amino acid sequence of the two fused monomers of avidin (dcAvd) (SEQ ID NO:2) with the locations of the wt  $\beta$ -strands indicated. The underlined ARK denotes the three first amino acids of wt avidin (SEQ ID NO:1). The artificial linkers GGSGGS (SEQ ID NO:3) and the monomer-monomer transition spacer SGG are highlighted with boxes. The part that is derived from cpAvd5→4 is underlined with the first bar and the part derived from cpAvd6→5 is underlined with the second bar. (B) Schematic illustration of the fused monomers of the wt structural dimer in dcAvd. The artificial linkers (GGSGGS) (SEQ ID NO: 3) that connect the original termini and the intermonomeric spacer (SGG) are circled. The left part is derived from cpAvd5→4 and the right part is derived from cpAvd6→5.

**Please amend the paragraph on page 6, lines 20-30 as follows:**

Figure 8. (A) Representation of the topology of the secondary structure elements of dcAvd. The original circularly permuted monomers are coloured red and blue. The peptide linkers connecting the old C- and N-terminus of avidin in cpAvd5→4 (blue) and cpAvd6→5 (red) as well as the linker which connects the cpAvds to form dcAvd are shown in black and green letters,

respectively. The locations of the mutagenised residues are shown by circles. The yellow circles refer to the modified biotin-binding residues. GGSGGS is disclosed as SEQ ID NO: 3. (B) Schematic representation of one possible quaternary structure of dcAvd and modified dcAvd. Colour codes as in (A). The peptide linker connecting the cpAvds is shown as a green tube. (C) Schematic representation of the biotin-binding residues mutagenised in this study and their interactions with biotin.

**Please amend the paragraph on page 7, line 20, as follows:**

Figure 14. Topology diagram of cpAvd4→3. GGSGGS is disclosed as SEQ ID NO: 3.

**Please amend the paragraph on page 7, lines 21-23, as follows:**

Figure 15. The topology diagram of dual chain avidin dcAvd54+43. In the figure, the red part shows the circularly permuted avidin 5→4 and the blue part the circularly permuted avidin 4→3. GGSGGS & SGGS are disclosed as SEQ ID NOS 3 & 30, respectively.

**Please amend the paragraph on page 7, lines 28-31 as follows:**

Figure 17. Dual chain avidin dcAvd54+43 amino acid sequence (SEQ ID NO:28). The signal peptide is underlined as well as the peptide linkers between N- and C-terminus of circularly permuted avidins and the SGGS (SEQ ID NO: 30) linker connecting 5→4 and 4→3 avidins.

**Please amend the paragraph on page 20, lines 20-35 as follows:**

In the next step in the case of cpAvd5→4 the region between beta strands 5 and 8 was PCR amplified with forward primer 54N1 5'-aag agg acc cag ccc acc tt -3' (SEQ ID NO:6) (coding also for the third amino acid, K, of avidin) and reverse primer 54C1 5'- gga gcc tcc gga gcc tcc ctc ctt ctg tgt gcg cag -3' (SEQ ID NO:7) (which inserts also the GGSGGS (SEQ ID NO: 3) linker after the beta strand eight). At the same time in a different tube the region between the beta strands 1 and 4 was PCR amplified with forward primer 54N2 5'- gga ggc tcc gga ggc tcc gcc aga aag tgc tcg ctg -3' (SEQ ID NO:8) (which codes also for the GGSGGS (SEQ ID NO: 3) linker, and the sequence is identical with the GGSGGS (SEQ ID NO: 3) coding part present in 54C1) and reverse primer 54C2 5'- tgggc aagct tca ctt gtt gat ggt gtt ttg-3' (SEQ ID NO:9)

(which contains a stop codon and a HindIII restriction site). These two PCR products were purified and used as a template in the subsequent PCR step, in which the fragments containing complementary regions (the GGSGGS (SEQ ID NO: 3) region) were combined and amplified with the terminal primers (54N1 and 54C2). This product was treated with HindIII and ligated into StuI and HindIII treated pFASTBAC1 derivative containing the signal sequence and the first two amino acids.

**Please amend the paragraph on page 21, lines 1-15 as follows:**

Similarly in the case of cpAvd6→5 the region between beta strands 6 and 8 was PCR amplified with forward primer 65N1 5'- aag tcc acc act gtc ttc acg -3' (SEQ ID NO:10) (which adds also the third amino acid, K, of avidin) and reverse primer 54C1, as previously described, (which inserts also the GGSGGS (SEQ ID NO: 3) linker after the beta strand eight). In a different tube the region between beta strands 1 and 5 was PCR amplified with forward primer 54N2, as previously described, (which codes also for the GGSGGS (SEQ ID NO: 3) linker, and the sequence is identical with the GGSGGS (SEQ ID NO: 3) coding part present in 54C1) and reverse primer 65C2 5'- agaca aagct tca ctc tga aaa ctt cca att g -3' (SEQ ID NO:11) (which contains a stop codon and a HindIII restriction site). These two PCR products were purified and used as a template in the subsequent PCR step, in which the fragments containing complementary regions (the GGSGGS (SEQ ID NO: 3)

region) were combined and PCR amplified with the terminal primers (65N1 and 65C2). This product was treated with HindIII and ligated into StuI and HindIII treated pFASTBAC1 derivative containing the signal sequence and the first two amino acids.

**Please amend the paragraph on page 32, lines 10-12 as follows:**

In this example two dcAvd-molecules are fused together tail-to-head via 12 amino-acid linker (GGSGSGSGSGSG) (SEQ ID NO: 31) to form a polypeptide with four binding sites for biotin. Other forms of linker may be also used.

**Please amend the paragraph on page 32, lines 19-27 as follows:**

pFASTBAC1 (cp6→5) was used as a template in PCR reaction with primers Single2.2 (5'- CCG GCA GAT CTA CCA CTG TCT TCA CGG GC) (SEQ ID NO:20) and Malooppi65.4 (5'-ATC CTC GGA TCC CGA TCC GGA ACC TCC CTC TGA AAA CTT C) (SEQ ID NO:21). The primer Single2.2 includes a BglII site. The primer Malooppi65.4 extends the sequence of cpAvd6→5 at the C-terminus with sequence GGSGSGS (SEQ ID NO: 32) and includes a BamHI site. The obtained PCR-product was extracted from 1% agarose gel, digested with BamHI and BglII and cloned to BamHI-digested pFASTBAC1(cpAvd5→4(no-stop))-plasmid. The obtained plasmid was sequenced and named pFASTBAC1(p54+p65looppi).

**Please amend the paragraph on page 32, line 28, to page 33, line 2 as follows:**

pFASTBAC1 (p54+p65-poisto) was used as a template in PCR reaction with primers MAlooppi54 (5'- GGC TCT GGT GGC TGG ATC CGG CTC TGG CAG CGG CAG GAC CCA GCC C) (SEQ ID NO:22) and A414 (5'- CTA CAA ATG TGG TAT GGC TG) (SEQ ID NO:23). The primer MAlooppi54 extends the sequence of cpAvd5→4 at the N-terminus with sequence GSGSGSG (SEQ ID NO: 33) and includes a BamHI site. The obtained PCR-product was extracted from 1% agarose gel and cloned to pGEM-T-easy vector using TA-cloning method. The obtained plasmid was sequenced and named pGemTeasy(p54looppi+p65).

**Please amend the paragraphs on page 38, line 18, to page 39, line 2 as follows:**

The circularly permuted avidin cpAvd4→3 was created by PCR method. First, a PCR reaction was carried out with primers 5,4N2 and cp34\_C1 AATTTAAGCT TATGTTACGG CTGTGATGTA G (SEQ ID NO:27) using avidin cDNA as a template. The PCR product was extracted from agarose gel after electrophoretic analysis. The second PCR was carried out with primers 5,4C1 and cp34\_C1 (AATTTAAGCT TATGTTACGG CTGTGATGTA G) (SEQ ID NO: 27). The PCR product was again extracted from agarose gel. PCR products from these reactions were combined in the third PCR reaction and they worked as megaprimers in this reaction.

The obtained DNA encoding for cpAvd4→3 was then cloned to a plasmid containing cp54 avidin (cpAvd5→4) and a SGGS (SEQ ID NO: 30) linker sequence. Restriction enzymes BamHI and HindIII were used in the cloning. The construct was confirmed by sequencing.

In cpAvd4→3, N- and C-terminus of avidin are joined by linker GGSGGS (SEQ ID NO: 3). Furthermore, part of the loop between beta strands 3 and 4 is removed, corresponding to residues 39ATSNEI44. In the circularly permuted avidin, the sequence starts before beta-strand 4 and ends after beta strand 3 (Figure 14).

**Please amend the paragraph on page 39, lines 23-26 as follows:**

Dual chain avidin dcAvd54+43 amino acid sequence (SEQ ID NO:28) is presented in figure 17. The signal peptide is underlined as well as the peptide linkers between N- and C-terminus of circularly permuted avidins and the SGGS (SEQ ID NO: 30) linker connecting 5→4 and 4→3 avidins.